Effects of adding fibrous feedstuffs to the diet of young pigs on growth performance, intestinal cytokines, and circulating acute-phase proteins

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Effects of adding fibrous feedstuffs to the diet of young pigs on growth performance, intestinal cytokines, and circulating acute-phase proteins[1]

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ABSTRACT: The effects of feeding different types of fiber to weanling pigs on growth performance, intestinal and liver cytokine expression, circulating acute-phase proteins, and IGF-I were evaluated. Intestinal tissue abundance of DNA, protein, and phosphorylated S6 kinase were also determined. Pigs (n = 120; initially 5.2 kg and 24 d of age) were randomly assigned to diets containing 1 of 4 fiber sources: 1) control diets containing no added fiber source, 2) diets containing 7.5% distillers dried grains with solubles (DDGS), 3) diets containing 7.5% soybean hulls, or 4) diets containing 7.5% citrus pulp. The experimental diets were fed for 4 wk in 2 phases (phase 1, wk 1 and 2; phase 2, wk 3 and 4). Intestinal tissue samples, liver samples, and blood samples were collected from a subset (n = 24; 6 pigs/treatment) of the pigs on d 7, and blood samples were collected from another subset (n = 24; 6 pigs/treatment) of pigs on d 28 of the experiment. Dietary treatment had no effect on ADG, ADFI, or G:F throughout the experiment. Likewise, pig BW variability (CV), plasma IGF-I, or the plasma concentration of the acute-phase proteins, α_1-acid glycoprotein, C-reactive protein, and haptoglobin, were not affected by dietary treatment. Real-time RT-PCR analysis revealed that on d 7, pigs fed DDGS had a greater ($P < 0.05$) relative abundance of the mRNA encoding IL-6, IL-1β, and IL-10 in ileum tissue than pigs fed all other diets. Diets containing DDGS had no effect on the relative abundance of tumor necrosis factor α or interferon-γ mRNA in ileum tissue on d 7. The d-7 mRNA expression of cytokines was not altered in jejunum, colon, or liver tissue by dietary treatment. Intestinal tissue protein content or jejunum and ileum DNA concentrations were not affected by diet. Western blot analysis found no effect of dietary treatment on the activation of S6 kinase in jejunum, ileum, or colon tissue on d 7. These results indicate that feeding 7.5% of a fiber source as DDGS, soybean hulls, or citrus pulp does not affect growth performance or circulating markers of inflammation in weanling pigs and that feeding DDGS increases the expression of both proinflammatory and antiinflammatory cytokines in intestinal tissue.

Key words: cytokine, fiber, nursery diet, pig

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INTRODUCTION

The consumption of dietary fiber has been shown to regulate systemic and intestinal inflammation. An inverse relationship between fiber intake and circulating acute-phase proteins has been observed in humans (Basu et al., 2006; Ma et al., 2006). Studies in young pigs have revealed that supplementing diets with fermentable fiber reduces the severity of *Salmonella* Typhimurium challenge (Correa-Matos et al., 2003), and feeding diets containing distillers dried grains with solubles (**DDGS**), which is high in hemicellulose, reduces the severity of intestinal lesions due to *Lawsonia intracellularis* infection (Whitney et al., 2006). Mechanistically, dietary fiber may alter the inflammatory response through regulating the expression of cytokines. Indeed, recent studies have shown that supplementing pig diets with a combination of 4 sources of dietary fiber increased colonic IL-6 expression (Pie et al., 2007). To date, there are no published data on the effects of different fiber types on the expression of cytokines or whether feeding fiber alters markers of systemic inflammation in pigs.

Inflammation via the action of cytokines has been found to regulate pathways involved in protein synthesis. Endotoxin challenge decreases the activation of S6

[1]Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. We thank Shari Steadham and Kellie Winter of the USDA-ARS Swine Odor and Management Unit for assistance with tissue collection.

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kinase (**S6K1**), a protein involved in protein translation initiation (Ruvinsky and Meyuhas, 2006) in skeletal muscle (Kimball et al., 2003; Lang and Frost, 2004). However, piglets infected with rotavirus enteritis have an increased level of activated S6K1 in intestinal tissue (Rhoads et al., 2007). There is little data available exploring the effect of dietary fiber on cytokine expression and subsequent effects on anabolic pathways and piglet growth.

Therefore, the objectives of this study were to determine the effects of different types of dietary fiber on pig growth, intestinal cytokine expression, and markers of systemic inflammation. Intestinal tissue DNA and protein content and the activation of intestinal S6K1 were also determined.

MATERIALS AND METHODS

Animals and Experimental Design

All animal care and handling procedures were approved by the Iowa State University Animal Care and Use Committee.

A total of 120 conventionally weaned, crossbred pigs (Pig Improvement Company, Lexington, KY; initial BW = 5.2 kg and 24 d of age) were used to evaluate the effects of dietary fiber addition to weanling pig diets on growth performance, intestinal cytokine gene expression, markers of systemic inflammation, intestinal tissue DNA and protein content, and the activation of S6K1 (Figure 1). No creep feed was provided to the pigs during the lactation period. The pigs were weaned and randomly assigned (stratified by ancestry, sex, and BW) to 4 dietary fiber treatments. Dietary fiber treatments consisted of the following: 1) control diets containing no added fiber source, 2) diets containing 7.5% DDGS, 3) diets containing 7.5% soybean hulls (**SBH**), or 4) diets containing 7.5 % dried citrus pulp. Each of the fiber sources was chosen to represent a different fiber type. Distillers dried grains with solubles are high in hemicellulose concentration (26% hemicellulose; 14% cellulose, on an as-fed basis), and SBH are high in cellulose (46% cellulose; 17% hemicellulose), whereas citrus pulp is high in pectin (45%) but lower in hemicellulose (5%) and cellulose (13%) compared with the other 2 sources of fiber. All diets were formulated to meet or exceed the established nutrient requirements for weanling pigs (NRC, 1998) and contained no antibiotics. The pigs were fed 2 dietary phases, with the pigs being switched to the second phase after the initial 2 wk (Table 1). The pigs were fed the same dietary fiber source throughout both dietary phases. All pigs had ad libitum access to feed and water through a self-feeder and a nipple waterer.

The pigs were reared in an environmentally controlled nursery facility in groups of 5 pigs per pen, with 6 replicate pens per dietary treatment. On d 7 of the experiment, 1 castrate male pig per pen closest to the average BW of the pen (n = 24; 6 pigs/dietary treatment) was removed from the experiment to obtain tissue and blood samples. Thereafter, there were 6 replicate pens with 4 pigs per pen for the remainder of the 4-wk experiment. At the completion of the experiment on d 28, blood samples were collected from 1 pig per pen from the castrate male pig closest to the average BW of the pen. The pigs and the feeders were weighed on d 7, the completion of phase 1, and at the end of the study to determine ADG, ADFI, G:F, and pen BW variability (CV). Records of pigs displaying signs of clinical illness and requiring therapeutic antibiotic treatment were maintained.

Sample Collection

Pigs were killed on d 7 postweaning for intestinal tissue cytokine analysis to capture some of the inflammation that can be associated with weaning (Pie et al., 2004), especially in pigs fed diets containing soy (McCracken et al., 1999). The expression of inflammatory cytokines has been shown to be elevated on d 8 postweaning (Pie et al., 2004); therefore, cytokine expression was measured when there would be a presumed increase in intestinal inflammation. Furthermore, an experiment conducted by Milo et al. (2002) showed that parenteral feeding of short-chain fatty acids (**SCFA**) to pigs resulted in an increase in intestinal cytokine expression on d 7. Therefore, it was also desirable to collect samples when there could be alterations in intestinal cytokines due to dietary fiber increasing the production of SCFA. Blood samples were collected on d 7 to relate any alterations in intestinal cytokines to markers of systemic inflammation. Blood samples were collected on d 28, because in human studies (Basu et al., 2006; Ma et al., 2006), several weeks of dietary fiber supplementation were necessary to decrease circulating acute-phase proteins. In addition, experiments conducted using the same facilities and similar genetics showed alterations in d-28 serum α_1-acid glycoprotein (**AGP**) in weanling pigs due to management schemes that were presumed to decrease systemic inflammation (Williams et al., 1997).

Pigs slaughtered for tissue collection on d 7 of the experiment were euthanized by captive bolt followed by exsanguination. Tissue samples were collected from the jejunum, ileum, colon, and liver. The tissue samples from the jejunum were collected from the middle third of the small intestine, and tissue samples from the ileum were collected from 15 cm cranial to the ileal-cecal junction. Colon samples were taken from a location 15 cm distal to the cecum. After dissection, the tissue samples were rinsed with ice-cold PBS and immediately placed into tubes containing RNAlater (Ambion Inc, Austin, TX) RNA stabilization solution or snap-frozen in liquid N. The tissue samples in RNAlater were initially stored overnight at 4°C and subsequently stored at –20°C pending mRNA analysis. Snap-frozen tissue samples were stored at –80°C until they were analyzed. Blood samples were collected from the vena cava before

Table 1. Composition of experimental diets (as-fed basis)

	Phase 1 (5.2 to 8.0 kg)				Phase 2 (8.0 to 14.5 kg)			
Item	Basal	DDGS	SBH	Citrus	Basal	DDGS	SBH	Citrus
Ingredient, %								
Corn	44.41	37.12	35.98	35.91	57.14	49.85	48.86	48.93
Soybean meal	25.10	24.94	24.92	26.23	23.39	23.23	23.05	24.20
Dried whey	20.00	20.00	20.00	20.00	10.00	10.00	10.00	10.00
Fiber source[1]	—	7.50	7.50	7.50	—	7.50	7.50	7.50
Menhaden fish meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vegetable oil	2.50	2.65	3.68	2.66	2.10	2.25	3.29	2.27
Dicalcium phosphate	1.22	0.97	1.22	1.21	1.72	1.47	1.72	1.72
Porcine plasma	1.00	1.00	1.00	1.00	—	—	—	—
Vitamin-mineral premix[2]	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Limestone	0.40	0.52	0.32	0.12	0.34	0.47	0.27	0.07
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys·HCl	0.18	0.16	0.17	0.16	0.18	0.16	0.17	0.17
DL-Met	0.13	0.10	0.14	0.14	0.07	0.05	0.09	0.09
L-Thr	0.06	0.03	0.06	0.06	0.05	0.02	0.06	0.06
Calculated analysis, %								
CP	21.2	22.6	21.4	21.6	19.4	20.7	19.4	19.6
Lys	1.44	1.46	1.45	1.44	1.27	1.29	1.28	1.26
Met	0.45	0.44	0.46	0.46	0.39	0.38	0.40	0.40
Thr	0.79	0.79	0.79	0.79	0.70	0.70	0.70	0.70
ME, Mcal/kg	3.38	3.38	3.38	3.38	3.38	3.38	3.38	3.38
Ca	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
P	0.81	0.81	0.80	0.81	0.83	0.83	0.82	0.82
NDF	7.27	9.46	11.00	8.03	8.15	10.33	11.86	8.89
Cellulose	2.65	3.30	5.57	3.25	2.77	3.42	5.67	3.35
Hemicellulose	4.39	5.65	4.96	4.12	5.12	6.38	5.69	4.86

[1]The added fiber sources consisted of no added fiber source (basal), 7.5% distillers dried grains with solubles (DDGS), 7.5% soybean hulls (SBH), or 7.5% dried citrus pulp (citrus). Phase 1 diets were fed wk 1 and 2, and phase 2 diets were fed wk 3 and 4 postweaning.

[2]The vitamin and trace mineral premix provided the following (per kg of diet): vitamin A, 11,023 IU; vitamin D_3, 2,756 IU; vitamin E, 55 IU; vitamin B_{12}, 55.0 μg; riboflavin, 16.535 mg; pantothenic acid, 44.1 mg; niacin, 82.7 mg; Zn, 150 mg; Fe, 175 mg; Mn, 60 mg; Cu, 17.5 mg; I, 2 mg; and Se, 0.3 mg.

captive-bolt stunning on d 7, and the pigs were sampled on d 28 at the completion of the experiment. The blood was collected into vacutainers containing EDTA (Becton Dickinson, Franklin Lakes, NJ) with recovered plasma stored at –80°C pending analysis.

RNA Isolation and Real-Time RT-PCR

Total RNA was isolated from intestinal, colon, and liver tissue samples using Trizol reagent (Invitrogen Inc., Carlsbad, CA) according to the protocol of the manufacturer, and the RNA pellets were resuspended in nuclease-free water. To eliminate possible genomic DNA contamination, the RNA samples were treated with a DNase I kit (DNA-free, Ambion Inc.) per the instructions of the manufacturer. The total RNA was quantified by measuring the absorbance at 260 nm using a NanoDrop ND-100 spectrophotometer (NanoDrop Technologies, Rockland, DE), and the purity was assessed by determining the ratio of the absorbance at 260 and 280 nm. All total RNA samples had 260/280 nm ratios above 1.8. Additionally, the integrity of the RNA preparations was verified by visualization of the 18S and 28S ribosomal bands stained with ethidium bromide after electrophoresis on 1.2% agarose gels (E-gel, Invitrogen Inc.). Total RNA (1 μg) was reverse-transcribed using a commercially available cDNA synthesis kit (iScript, BioRad Laboratories, Hercules, CA).

Real-time PCR detection of cytokine mRNA was conducted utilizing the SYBR Green assay. All of the primers used for real-time PCR, except for IL-1β (Hyland et al., 2006), were newly designed using Primer3 software (Rozen and Skaletsky, 2000) and are presented in Table 2. Amplification was carried out in a total volume of 25 μL containing 1X iQ SYBR Green Supermix (BioRad), forward and reverse primers (0.1 μg/μL), and 1 μL of the cDNA reaction. After an initial 5-min denaturation step at 95°C, the reactions were cycled 40 times under the following parameters: 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. Optical detection was carried out at 72°C. At the end of the PCR, melt curve analysis was conducted to validate the specificity of the primers. Thermal cycling conditions and real-time detection were conducted using an Opticon Chromo 4 real-time PCR detection system (BioRad). A nontemplate control was used with every assay, and all determinations were performed in duplicates. External cDNA standards were constructed by cloning the corresponding RT-PCR product into a PCR 4-TOPO vector (Invitrogen), and the resultant plasmids were sequenced at the Iowa State University DNA facility for verification. Serial dilutions of a known amount of plasmid containing the cDNA

Table 2. Porcine-specific primers used for real-time PCR

Gene	Primer sequences (5′ → 3′)[1]	Amplicon length (bp)	Accession number and reference
IGF-I	(S) GCCTCAGGGCTCAATTCATA (AS) GGTGCCTGACAAGGACGTAT	137	X64400
IL-6	(S) GCCACCTCAGACAAAATGCT (AS) TCTGCCAGTACCTCCTTGCT	143	NM_214399
IL-1β	(S) CCTCCTCCCAGGCCTTCTGT (AS) GGGCCAGCCAGCACTAGAGA	178	M86725 (Hyland et al., 2006)
IL-10	(S) GCATCCACTTCCCAACCA (AS) CTTCCTCATCTTCATCGTCAT	445	NM_214041.1
IFN-γ[2]	(S) CAGAGCCAAATTGTCTCCTTCTAC (AS) TCTCTGGCCTTGGAACATAG	284	NM_213948.1
SOCS3[3]	(S) AGATCCCTCTGGTGTTGAGC (AS) CGTTGACTGTTTTCCGACAG	77	AY785557
TNFα[4]	(S) CCCAAGGACTCAGATCATCG (AS) ATACCCACTCTGCCATTGGA	101	x57321
β-actin	(S) GGACCTGACCGACTACCTCA (AS) GCGACGTAGCAGAGCTTCTC	115	u07786

[1]S = sense primer; AS = antisense primer.
[2]IFN-γ = interferon-γ.
[3]SOCS3 = suppressor of cytokine signaling 3.
[4]TNFα = tumor necrosis factor α.

of interest were included on each 96-well plate. The abundance of each gene product was calculated by regressing against the standard curve generated in the same reaction with their respective plasmids. The RNA abundance values for each sample were normalized to β-actin. The mRNA expression of β-actin was not affected by dietary treatment in any of the tissues.

Plasma Acute-Phase Proteins and IGF-I

Plasma AGP was measured by using a porcine-specific radial immunodiffusion kit (Cardiotech Services Inc., Louisville, KY). The intra- and interassay CV were 4%, and the range of detection was 50 to 1,500 μg/mL. Plasma concentrations of haptoglobin were measured using a commercially available ELISA kit that is porcine-specific (ALPCO Diagnostics, Salem, NH). Plasma samples were diluted 1:10,000 with the diluent provided with the kit before analysis. The haptoglobin ELISA has a range of detection from 6.25 to 400 ng/mL. The abundance of C-reactive protein in the plasma samples was measured using a porcine-specific ELISA kit (Immunology Consultants Laboratory Inc., Newberg, OR). The plasma samples were diluted 1:2,000 with the assay buffer before to the ELISA. The C-reactive protein ELISA has a range of detection from 6.25 to 200 ng/mL. The intrassay CV for the haptoglobin ELISA was 5.29%, whereas the interassay CV was 9.14%. Based on 2 assays, the intra- and interassay CV for the C-reactive protein ELISA were less than 10%.

Plasma IGF-I concentrations were determined using a commercially available kit (Active IGF-I ELISA, Diagnostic Systems Laboratories Inc., Webster, TX). The plasma samples were pretreated with the solutions provided with the kit to separate IGF-I from binding proteins. The samples were analyzed in duplicate using the protocol of the manufacturer. The assay was validated for porcine plasma by spiking a pooled porcine plasma sample with known quantities of standard and by serial dilution of the pooled plasma sample. Based on these 2 assays, the intra- and interassay CV were less than 9%.

Intestinal Tissue Protein Extraction and DNA Determination

Snap-frozen samples of jejunum, ileum, and colon tissue were homogenized in T-PER Mammalian Protein Extraction Reagent (Pierce, Rockford, IL) supplemented with protease (Roche, Indianapolis, IN) and phosphatase (Sigma Chemical Co., St. Louis, MO) inhibitors. The tissue homogenates were centrifuged at 10,000 × *g* for 5 min at room temperature, and the supernatants were used for Western blot analysis. Intestinal tissue homogenate protein concentrations were determined using the Bicinchoninic Acid Assay (Pierce) method using BSA as a standard. The DNA concentrations of the intestinal tissue homogenates were determined using the Hoechst 33258 dye (Sigma) method (Labarca and Paigen, 1980). Calf thymus DNA (Sigma) was used for the standards for DNA determinations.

Western Blot Analysis of Intestinal Phosphorylated S6K1

Intestinal protein extracts (50 μg/lane) were fractionated by SDS-PAGE using 4 to 12% bis/Tris gels and transferred to nitrocellulose membranes (BioRad). The membranes were blocked for 1 h at room temperature with 5% (wt/vol) nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20 (Sigma). Then the membranes were incubated overnight at 4°C with a rabbit

antiphospho-p70 S6K1 (Thr389) antibody (Zymed, South San Francisco, CA) diluted 1:250 in blocking solution. After washing with Tris-buffered saline containing 0.1% Tween 20, the membrane was incubated with a horseradish peroxidase labeled secondary antibody (Zymed) diluted 1:5,000 in blocking solution. The reaction complexes were visualized using a chemiluminescent detection kit (Pierce). The membranes were exposed to x-ray film, and the films were scanned and densitometry analysis was conducted using a BioRad Fluor-S MultiImager. The blots were then stripped using Restore Western Blot Stripping Buffer (Pierce) for 15 min at 37°C. Glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) was detected using a mouse anti-GAPDH monoclonal antibody (BioChain Institute Inc., Hayward, CA) diluted at 1:2,000 in blocking solution. The GAPDH reaction complexes were visualized as described above. Band density for phosphorylated S6K1 was normalized against the GAPDH loading control.

Statistical Analysis

All data were analyzed by single-factor ANOVA using the GLM procedure (SAS Inst. Inc., Cary, NC). Pen served as the experimental unit for the growth performance data, and pig served as the experimental unit for the mRNA, protein, and plasma data. When ANOVA indicated a significant ($P < 0.05$) difference, the means were separated using the Student-Newman-Keuls multiple range test. The means were also separated, and statistical trends were discussed, when ANOVA indicated $P < 0.10$. The frequency data for the number of pigs requiring therapeutic injectable antibiotic treatment were analyzed using adjusted χ^2 analysis in the FREQ procedure of SAS.

RESULTS

Growth Performance

Adding 7.5% of a fibrous feedstuff had no effect on ADG, ADFI, or G:F in weanling pigs during any time in which growth performance was monitored (Table 3). The addition of fiber sources to the diets had no effect on pen BW variability or the proportion of pigs requiring therapeutic treatment with antibiotics. There were no pig mortalities throughout the course of the experiment.

Intestinal and Hepatic Tissue Cytokine mRNA expression

Real-time PCR analysis revealed that adding 7.5% DDGS to the diet increased ($P < 0.05$) the relative abundance of IL-6 and IL-1β mRNA in ileum tissue (Table 4). Likewise, feeding pigs diets containing DDGS increased ($P < 0.05$) the mRNA expression of IL-10, but diet had no effect on the mRNA expression of tumor necrosis factor α (**TNFα**) or interferon-γ (**IFN-γ**). There was an overall treatment trend ($P < 0.07$) for dietary treatment on the expression of suppressor of cytokine signaling 3 (**SOCS3**) in ileum tissue, because pigs fed DDGS had a greater ($P < 0.05$) relative abundance of SOCS3 mRNA when compared with pigs fed the control diet or pigs fed citrus. There was no effect of dietary treatment on IGF-I gene expression in ileum tissue. Dietary fiber treatment did not affect the mRNA expression of IL-6, IL-1β, TNFα, or SOCS3 in jejunum, colon, or liver tissue.

Plasma Acute-Phase Proteins and IGF-I

The addition of 7.5% of a dietary fiber source did not affect circulating concentrations of AGP, C-reactive protein, or haptoglobin in pigs sampled on d 7 or 28 of the experiment (Table 5). Likewise, plasma IGF-I concentrations on d 7 or 28 were not affected by dietary treatment.

Intestinal Tissue DNA and Protein Content and S6K1 Activation

Concentrations of DNA and protein in jejunum and ileum tissue on d 7 were not affected by dietary fiber source (Table 6). There was a trend ($P < 0.08$) for an overall treatment effect for colon tissue DNA concentrations. When compared with pigs fed the control diet or pigs fed citrus, pigs fed diets containing DDGS had decreased ($P < 0.05$) colon tissue DNA concentrations. Colon tissue protein concentration was not affected by diet. Adding 7.5% of a fibrous feedstuff to the diet was not found to alter the activation of S6K1 in jejunum, ileum, or colon tissue on d 7 (Figure 2).

DISCUSSION

The addition of 7.5% of a fiber source as DDGS, SBH, or citrus pulp did not appear to affect overall animal health status, because there were no differences observed for animals requiring antibiotic treatment, plasma acute-phase proteins, or plasma IGF-I. In addition, we showed that adding fiber to weanling pig diets did not affect growth performance or feed intake. Our data support the results of a previous study conducted by Whitney and Shurson (2004), who concluded that DDGS can be included at levels up to 10% of the diet without depressing the growth performance or feed intake of weanling pigs. Likewise, it was determined that adding 7.5% of a combined fiber source (sugar beet pulp, inulin, and lactulose) had no effect on the feed intake of weanling pigs (Pie et al., 2007). Kornegay et al. (1995) showed that adding 8% SBH to starter pig diets had no effect on ADG or ADFI. More recently, data from Bikker et al. (2006) suggested that including 4.5% sugar beet pulp has no effect on the growth performance of weanling pigs as long as CP levels are adequate. Collectively, these results indicate that several different types of fiber can be successfully fed to weanling pigs at levels

Table 3. Effects of dietary fiber source on the growth performance of weanling pigs

	Fiber source[1]					
Item	Control	DDGS	Soy hulls	Citrus pulp	SEM	*P*-value
ADG, g						
wk 1	125.7	133.8	144.6	109.8	10.6	0.16
Phase 1[2]	184.6	190.1	215.9	169.7	13.7	0.13
Phase 2	453.4	475.3	480.7	437.7	18.9	0.37
wk 1 to 4	319.1	332.6	348.9	303.6	13.7	0.15
ADFI, g						
wk 1	139.3	138.1	153.8	122.5	12.5	0.40
Phase 1	222.7	220.0	251.3	204.6	16.6	0.28
Phase 2	670.9	690.4	703.5	627.3	24.9	0.19
wk 1 to 4	446.8	455.2	477.4	416.0	19.5	0.19
Gain:feed, g/g						
wk 1	0.901	0.968	0.940	0.892	0.04	0.40
Phase 1	0.829	0.863	0.859	0.829	0.04	0.49
Phase 2	0.676	0.688	0.685	0.697	0.02	0.80
wk 1 to 4	0.752	0.776	0.772	0.763	0.02	0.45
BW, kg						
Initial	5.22	5.21	5.26	5.30	0.29	0.99
wk 4	14.34	14.57	15.21	13.93	0.65	0.58
BW variation, CV						
Initial	4.65	3.63	3.38	3.57	1.0	0.82
wk 4	14.63	14.65	7.50	8.71	2.9	0.20
% Treated[3]	6.67	10.00	16.67	6.67	—	0.53

[1]Weanling pigs (n = 120; 6 pens/treatment) were fed diets containing no added fiber source (control) or diets containing 7.5% distillers dried grains with solubles (DDGS), 7.5% soybean hulls, or 7.5% citrus pulp for 4 wk after weaning.
[2]Phase 1 diets were fed wk 1 and 2, and phase 2 diets were fed wk 3 and 4.
[3]Percentage of pigs requiring treatment with injectable antibiotics. Data were analyzed using χ^2 analysis.

between 5 and 10% without depressing growth performance.

Despite the lack of an effect of dietary treatment on growth performance or circulating acute-phase proteins, it was showed that adding 7.5% DDGS to the diet induced an upregulation of IL-6 and IL-1β mRNA in ileum tissue. The increased expression of these cytokines was localized to the ileum, and no change in cytokine expression was found in jejunum, colon, or liver tissues. In contrast, Pie et al. (2007) showed that feeding a mixed fiber source increased the relative abundance of IL-6 mRNA in colon tissue on d 4 postweaning. The discrepancies between studies could be due to difference in day of sampling (d 4 vs. 7) or that different fiber sources were used. Interestingly, an overall treatment trend ($P < 0.07$) was noted for the mRNA expression of SOCS3 in ileum tissue. The expression of SOCS3 is regulated by IL-6 (Starr et al., 1997), and SOCS3 serves as a negative feedback mechanism for IL-6 signaling in intestinal epithelial cells (Wang et al., 2003) and colon tissue (Suzuki et al., 2001). Perhaps a greater sample size of pigs would have resulted in a statistically significant ($P < 0.05$) increase in SOCS3 mRNA expression.

Mechanistically, dietary fiber may alter intestinal cytokine expression via changing the milieu of SCFA produced by microflora that are present in the distal ileum and throughout the colon (Drochner et al., 2004). It has been demonstrated that parenterally feeding piglets a mixture of SCFA (containing acetate, proprionate, and butyrate) increased the abundance of IL-1β and IL-6 in the ileum (Milo et al., 2002). In Milo et al. (2002), it is interesting that the increase in these cytokines was localized to the ileum and that there was no increase in intestinal TNFα expression or circulating cytokine concentrations. Furthermore, previous data collected in our laboratory (Weber and Kerr, 2006) showed that sodium butyrate directly increased the expression of SOCS3 and IL-10 in porcine lymphocytes. Thus, it could be proposed that dietary DDGS may increase intestinal cytokine expression through increasing intestinal SCFA abundance.

Regarding the different fiber sources used in the current study, it would be presumed that the citrus pulp fiber would be more fermentable than the fiber contained in DDGS or SBH (Sunvold et al., 1995). Therefore, if increased SCFA production was responsible for increasing ileal cytokine expression, it is surprising that citrus did not alter cytokine expression in intestinal tissue as shown in pigs fed DDGS. Based on fermentation characteristics, it would be unlikely that dietary SBH would alter intestinal cytokines via an increase in SCFA. The lack of an effect of SBH on intestinal cytokines further supports the findings of Pie et al. (2007), in which a mix of fermentable fiber increased colonic IL-6. Pie et al. (2007) included cellulose to the control diet used in their study, and our data indicate that the cellulose included in their control diet would have had little effect on intestinal cytokine expression.

Table 4. Effects of dietary fiber source on the relative abundance of cytokine mRNA in ileum, jejunum, colon, and liver tissue on d 7 of the experiment

	Fiber source[1]					
Item	Control	DDGS	Soy hulls	Citrus pulp	SEM	*P*-value
Jejunum mRNA						
IL-6	0.002	0.003	0.004	0.003	0.001	0.72
IL-1β	0.019	0.031	0.023	0.029	0.008	0.74
TNFα[2]	0.003	0.003	0.003	0.005	0.001	0.48
SOCS3[3]	0.022	0.016	0.019	0.015	0.004	0.55
Ileum mRNA						
IL-6	0.004^{x}	0.017^{y}	0.004^{x}	0.006^{x}	0.003	0.04
IL-1β	0.020^{x}	0.061^{y}	0.013^{x}	0.020^{x}	0.012	0.03
TNFα	0.003	0.003	0.001	0.002	0.001	0.48
SOCS3	0.010^{x}	0.016^{y}	0.012^{x,y}	0.008^{x}	0.002	0.07
IFN-γ	0.003	0.007	0.003	0.007	0.002	0.25
IL-10	0.005^{x}	0.017^{y}	0.005^{x}	0.008^{x}	0.003	0.05
IGF-I	0.097	0.057	0.096	0.068	0.020	0.42
Colon mRNA						
IL-6	0.008	0.009	0.010	0.007	0.002	0.71
IL-1β	0.087	0.074	0.010	0.064	0.020	0.53
TNFα	0.003	0.002	0.002	0.002	0.001	0.76
SOCS3	0.016	0.017	0.017	0.018	0.003	0.97
Liver mRNA						
IL-6	0.008	0.010	0.005	0.004	0.003	0.36
IL-1β	0.016	0.004	0.005	0.005	0.005	0.28
TNFα	0.009	0.004	0.003	0.004	0.002	0.14
SOCS3	0.034	0.033	0.038	0.025	0.008	0.69

^{x,y}Means with different superscripts within each row differ ($P < 0.05$).

[1]Weanling pigs (n = 120; 6 pens/treatment) were fed diets containing no added fiber source (control) or diets containing 7.5% distillers dried grains with solubles (DDGS), 7.5% soybean hulls, or 7.5% citrus pulp. Tissue samples were harvested from a subset of pigs (n = 6 pigs/treatment) on d 7 after weaning for mRNA expression analysis. The mRNA abundance is normalized to the mRNA expression of β-actin.

[2]TNFα = tumor necrosis factor α.

[3]SOCS3 = suppressor of cytokine signaling 3.

An alternative explanation for the increased expression of ileal cytokines found in pigs fed DDGS is that yeast cell wall components remaining from the ethanol fermentation process may be acting to alter mucosal immunity. Indeed, recent data suggest that yeast cell wall β-glucan alters intestinal cytokine mRNA expression in weanling pigs (Eicher et al., 2006). Futhermore, Pie et al. (2007) conducted stepwise regression analysis and showed no significant relationship between colonic SCFA content and the expression of IL-1β or IL-

Table 5. Effects of dietary fiber source on plasma acute-phase proteins and IGF-I

	Fiber source[1]					
Item	Control	DDGS	Soy hulls	Citrus pulp	SEM	*P*-value
α_1-AGP,[2] μg/mL						
d 7	803	735	704	626	75	0.43
d 28	1,085	1,105	942	1,003	81	0.47
CRP,[3] μg/mL						
d 7	16.6	19.4	17.1	17.1	4.4	0.97
d 28	27.5	20.2	33.5	29.4	4.7	0.28
Haptoglobin, μg/mL						
d 7	525	813	506	679	215	0.72
d 28	488	605	552	492	195	0.97
IGF-I, ng/mL						
d 7	50.9	58.2	46.1	37.3	7.9	0.32
d 28	158.4	153.2	200.9	125.7	22.6	0.16

[1]Weanling pigs (n = 120; 6 pens/treatment) were fed diets containing no added fiber source (control) or diets containing 7.5% distillers dried grains with solubles (DDGS), 7.5% soybean hulls, or 7.5% citrus pulp. Plasma samples were harvested from a subset of pigs (n = 6 pigs/treatment; 1 pig/pen) on d 7 and 28 after weaning for the analysis of acute-phase proteins and IGF-I.

[2]α_1-AGP = α1-acid glycoprotein.

[3]CRP = C-reactive protein.

Table 6. Effects of dietary fiber source on intestinal tissue DNA and protein content on d 7 of the experiment

	Fiber source[1]					
Item	Control	DDGS	Soy hulls	Citrus pulp	SEM	*P*-value
Jejunum						
DNA, mg/g	0.88	0.99	1.32	1.59	0.28	0.30
Protein, mg/g	54.4	54.6	58.6	52.0	4.7	0.80
Ileum						
DNA, mg/g	4.63	4.08	3.48	5.17	0.63	0.29
Protein, mg/g	49.5	50.3	43.6	47.4	3.9	0.61
Colon						
DNA, mg/g	1.18[x]	0.82[y]	1.07[x,y]	1.34[x]	0.13	0.08
Protein, mg/g	25.9	25.9	33.9	36.8	4.3	0.19

[x,y]Means with different superscripts within each row differ ($P < 0.05$).
[1]Weanling pigs (n = 120; 6 pens/treatment) were fed diets containing no added fiber source (control) or diets containing 7.5% distillers dried grains with solubles (DDGS), 7.5% soybean hulls, or 7.5% citrus pulp. Intestinal tissue samples were harvested from a subset of pigs (n = 6 pigs/treatment; 1 pig/pen) on d 7 of the experiment.

6. Taken together, these findings indicate that other factors, in addition to SCFA, may alter the expression of cytokines in response to dietary DDGS.

Another alternative mechanism for the increase in intestinal cytokine expression with DDGS could be the increase in dietary S intake. Our laboratory recently had several fibrous feedstuffs analyzed for total S content. The sulfur content of DDGS was shown to be 6,886 ppm, whereas the S content of SBH, beet pulp, and corn grain was 1,414 ppm, 1,775 ppm, and 1,132 ppm, respectively. Relative to other feedstuffs, a greater portion of the S in DDGS is believed to be in the sulfate form, because sulfuric acid is added during the fermentation process. The increased sulfate intake in pigs consuming DDGS would likely provide more substrates for sulfate-reducing bacteria, which are present in the porcine intestine (Butine and Leedle., 1989) to produce greater concentrations of hydrogen sulfide. Recently, hydrogen sulfide has been shown to directly increase cytokine production in human macrophages (Zhi et al., 2007) and increase systemic cytokines in response to sepsis in mice (Zhang et al., 2007). In addition, the abundance of sulfate-reducing bacteria was found to be greater in fecal samples collected from infants with celiac disease (Collado et al., 2007), which further indicates a relationship between S and intestinal inflammation. Further research is necessary to clarify the relationship between dietary S and intestinal cytokine expression.

The biological ramifications of increased ileal tissue cytokine expression remain to be determined. Increased IL-6 expression and downstream signaling via signal transducer and activator of transcription 3 (STAT3) is associated with increased inflammation in ileitis (Mitsuyama et al., 2006) and colitis (Suzuki et al., 2001), yet antiinflammatory compounds such as n-3 fatty acids

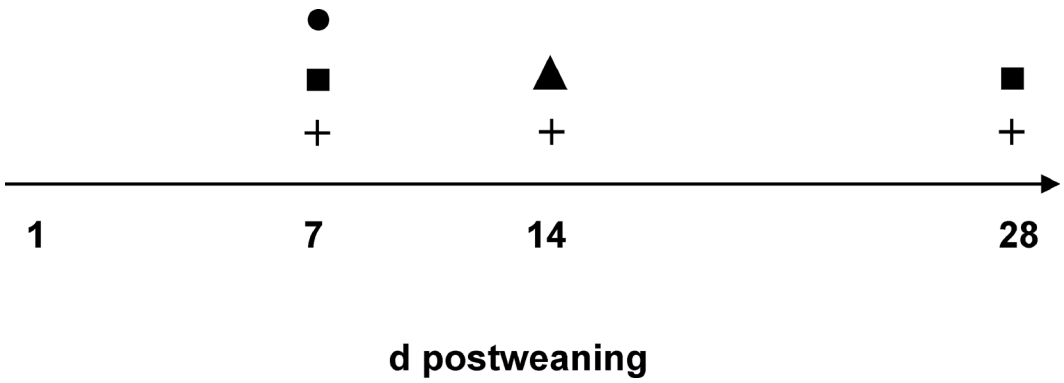

Figure 1. Experimental sampling protocol. For the growth performance and ADFI data, 120 pigs (24 pens containing 5 pigs/pen) were used. This resulted in 6 replicate pens for each of the 4 dietary fiber treatments: 1) control diets containing no added fiber source, 2) diets containing 7.5% distillers dried grains with solubles, 3) diets containing 7.5% soybean hulls, or 4) diets containing 7.5% dried citrus pulp. Body weight and ADFI (+) were measured on d 7, 14, and 28. Intestinal tissues (●) and blood samples (■) were collected from 1 pig/pen (n = 6 pigs/treatment) on d 7, and blood samples were collected from 1 pig/pen on d 28. All pigs were switched (▲) to phase 2 diets on d 14.

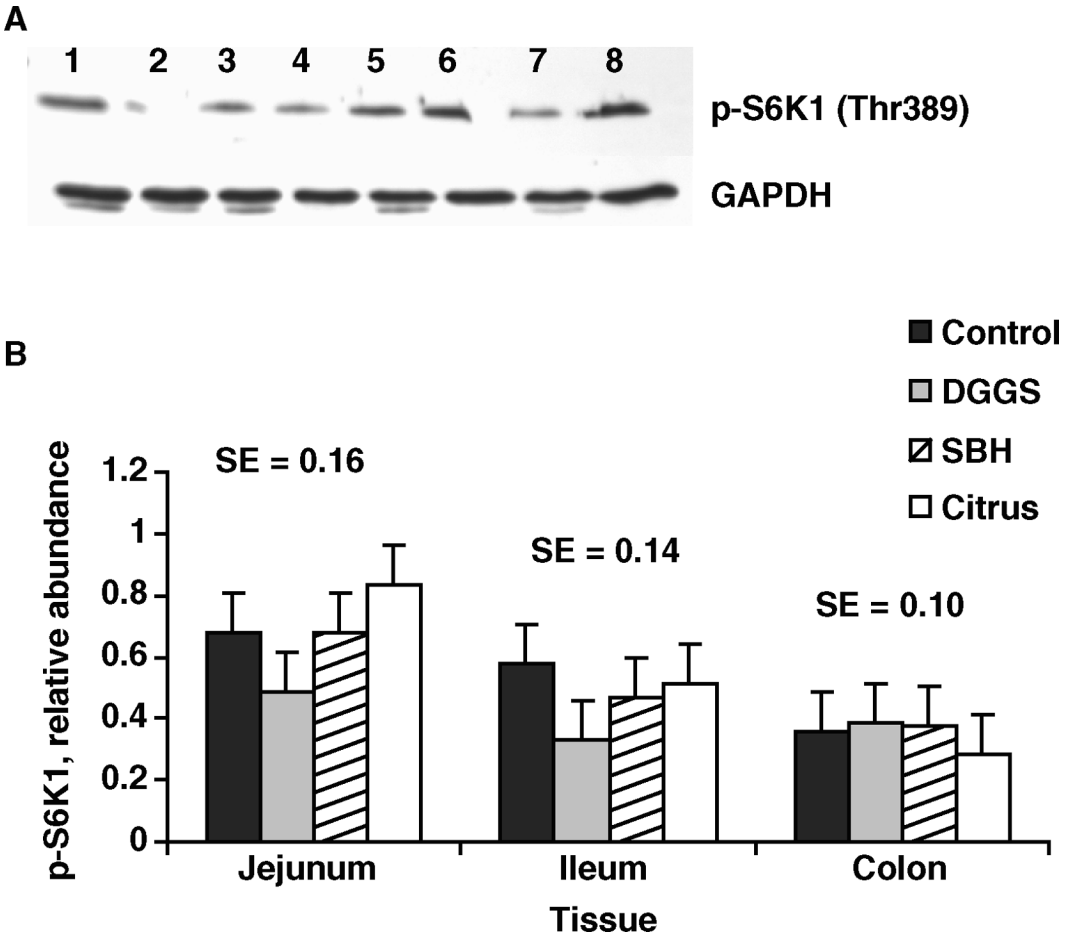

Figure 2. Western blot analysis of the phosphorylated S6 kinase (p-S6K1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in intestinal tissues of weanling pigs on d 7 of the experiment. (A) Representative picture of Western blot of porcine intestinal tissue. Lanes 1 and 5 are intestinal samples from pigs fed the control diet, lanes 2 and 6 are from pigs fed a diet containing 7.5% distillers dried grains with solubles (DDGS), lanes 3 and 7 are samples from pigs fed 7.5% soybean hulls (SBH), and lanes 4 and 8 are samples from pigs fed 7.5% citrus pulp (citrus). (B) Density analysis (normalized to GAPDH) of the relative abundance of p-S6K1 in jejunum, ileum, or colon tissue of weanling pigs fed control diets or diets containing 7.5% DDGS, 7.5% SBH, or 7.5% citrus for 7 d. n = 6 pigs/treatment. No significant differences were found.

are reported to increase intestinal IL-6 expression (Hsu et al., 2006). Furthermore, transgenic mice created to produce endogenous n-3 fatty acids are protected from colitis (Hudert et al., 2006). Recent studies conducted in rodents showed that IL-6 is responsible for growth retardation in response to intestinal inflammation (Sawczenko et al., 2005). In Sawczenko et al. (2005), administering an antibody directed against IL-6 reversed the growth depression, increased circulating IGF-I, and decreased circulating C-reactive protein in mice with experimentally induced colitis. In our study, circulating IGF-I concentrations, ileal IGF-I expression, or plasma C-reactive protein concentrations were not altered in pigs fed DDGS. This suggests that the increase in ileal inflammatory cytokine expression in response to DDGS does not lead to a systemic inflammatory response and subsequent growth suppression.

The expression of intestinal IL-1β has been shown to be upregulated during bacterial infection (Hyland et al., 2006). However, IL-1β has also been implicated as necessary for intestinal repair after ischemia (Shifflett et al., 2004). Both IL-6 and IL-1β mRNA are upregulated shortly after weaning (Pie et al., 2004), perhaps suggesting a role in intestinal adaptation and remodeling due to stress and dietary changes. These contradictory roles for inflammatory cytokines within the intestine make it difficult to ascertain whether increased cytokine expression in response to DDGS is deleterious or beneficial. Likewise, further research is necessary to clarify whether the reduction in the prevalence and severity of intestinal lesions in ileitis-challenged pigs fed 10% DDGS (Whitney et al., 2006) is associated with alterations in cytokine expression.

To further evaluate the effect of dietary fiber on cytokine expression in ileum tissue, IL-10 and IFN-β mRNA were measured. It was shown that DDGS increased the expression of the antiinflammatory cytokine, IL-10, but not IFN $-\beta$. This, along with the tendency toward an

increase in SOCS3 gene expression, is indicative of a T-helper type 2 immune response (Egwuagu et al., 2002). The lack of an increase in circulating acute-phase proteins or decrease in ileum IGF-I in the present experiment may indeed be attributed to the antiinflammatory roles of IL-10 and SOCS3 and their roles in immune response polarization toward antibody production as opposed to inflammation. It is also interesting that IL-6 has been shown to promote IgA secretion (Mora et al., 2006), which further indicates that the cytokine response to DDGS may be a T-helper type 2 response. However, further research is necessary to determine whether feeding a feedstuff such as DDGS increases intestinal antibody synthesis.

As a measure of intestinal tissue hyperplasia, the intestinal DNA content was determined. Besides an overall treatment trend ($P < 0.08$) for differences in colon tissue DNA content, there were no effects of dietary fiber source on intestinal tissue DNA content. This finding is not in agreement with Jin et al. (1994), in which it was shown that pigs fed a diet containing 10% wheat straw for 14 d had a decreased abundance of DNA in jejunum and ileum tissue. The different findings between the studies may be due to the shorter duration of fiber consumption (7 vs. 14 d) in the current study. The trend toward a decrease in ileum tissue DNA content in pigs fed DDGS is interesting in the context of an increase in cytokine expression. Intestinal inflammation has been shown to increase intestinal crypt cell hyperplasia (MacDonald, 1992) and would presumably lead to an increase in intestinal tissue DNA content.

It is interesting that feeding DDGS or other fiber sources did not alter the activation of intestinal phosphorylated S6K1 given that there was an increase in cytokine expression. A recent study conducted in young pigs demonstrated that challenge with rotavirus increased the phosphorylation of S6K1 (Rhoads et al., 2007). Likewise, sepsis (Lang and Frost, 2004) and endotoxin (Lang and Frost, 2005) have been shown to inhibit muscle S6K1 activation in skeletal muscle, but the roles of cytokines in intestinal S6K1 signaling have not been determined. Both sepsis (Lang and Frost, 2004) and endotoxin (Lang and Frost, 2005) increased circulating concentrations of inflammatory cytokines, but in our study plasma markers of inflammation were not altered by diet. It was initially hypothesized that supplementing the diet with fiber would increase the phosphorylation of S6K1. Increases in intestinal protein synthesis (Pirman et al., 2007) in pectin-supplemented rats and feeding pigs diets containing 40% alfalfa meal increased total gastrointestinal tract weight (Anugwa et al., 1989). Further research is necessary to determine the role of dietary fiber and cytokines in the intestinal mammalian target of rapamycin/S6K1 pathway.

In summary, it was determined that adding 7.5% DDGS, SBH, or citrus pulp had no effects on growth performance or markers of systemic inflammation. It was shown that adding 7.5% DDGS to weanling pig diets upregulated the expression of IL-6 and IL-1β mRNA in ileum tissue. Feeding diets containing DDGS also increased the abundance of IL-10 but had no effect on IFN-β mRNA. Soybean hulls or citrus pulp did not affect intestinal cytokine expression. Lastly, the feeding of fiber to weanling pigs did not alter the activation of S6K1 in intestinal tissue. These data indicate that the alterations in constitutive intestinal cytokine expression are not necessarily associated with changes in growth performance or systemic inflammation and that 7.5% DDGS, SBH, or citrus pulp can successfully be fed to weanling pigs.

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